OPTION A: ANALYTICAL CHEM
A7 – CHROMATOGRAPHY

IB Chemistry

TAD06

Mr. Martin Brakke

A6 – Atomic Absorption Spectroscopy

- A.7.1 State the reasons for using chromatography. (1)
  - *The qualitative and quantitative aspects of chromatography should be outlined.*
- A.7.2 Explain that all chromatographic techniques involve adsorption on a stationary phase and partition between a stationary phase and a mobile phase. (3)
  - *Components in a mixture have different tendencies to adsorb onto a surface or dissolve in a solvent. This provides a means of separating the components of a mixture.*
- A.7.3 Outline the use of paper chromatography, thin-layer chromatography (TLC) and column chromatography. (2)
  - *An outline of the operation for each technique will be assessed. This should include an understanding and calculation of Rf values where relevant. Students should be aware that, in some instances, paper chromatograms may need to be developed, for example, in the separation of sugars.*

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Justifying Chromatography

A.7.1 State the reasons for using chromatography. (1)

- The presence harmful impurities or contaminants in drugs, foods, and water is a concern of regulating organizations such as the FDA (Food and Drug Administration).
- The purity of a substance must be determined
  - If it can be separated into two or more distinct components (analytes) then further tests can be run
  - Preparative Chromatography
    - To separate the components for further use (purification)
  - Analytical Chromatography
    - To identify trace amounts (and relative proportions) of analytes

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Phases of Chromatography

A.7.2 Explain that all chromatographic techniques involve adsorption on a stationary phase and partition between a stationary phase and a mobile phase. (3)

- A sample is placed in a liquid or solid **stationary phase** and a liquid or gaseous **mobile phase** is passed over it
- In our example of a Sharpie® spotted on filter paper and placed vertically in a dish of ethanol.
  - **Stationary Phase** – the spotted sample
  - **Mobile Phase** – the ethanol running up the paper via capillary action
  - **Elution** – the process of materials migrating at different speeds depending on solubility
- This applies to all chromatography techniques

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Chromatography Techniques

Technique	Stationary Phase	Mobile Phase	Format	Mechanism of Separation
Paper Chromatography	Paper (cellulose)	Liquid	Flat	Partition
Thin Layer Chromatography (TLC)	Silica, cellulose	Liquid	Flat	Adsorption or partition
Gas-Liquid Chromatography (GLC)	Liquid	Gas	Column	Partition
High-Performance Liquid Chromatography (HPLC)	Solid	Liquid	Column	Modified partition

- Classified according to whether the separation takes place
  - On a flat surface or in a column
  - In the gas or liquid phase
  - If the stationary phase is solid or liquid
  - Interaction between the mobile and stationary phase
    - **Adsorption** – solute held on the surface of the stationary phase
    - **Partition** – solute split between two liquids

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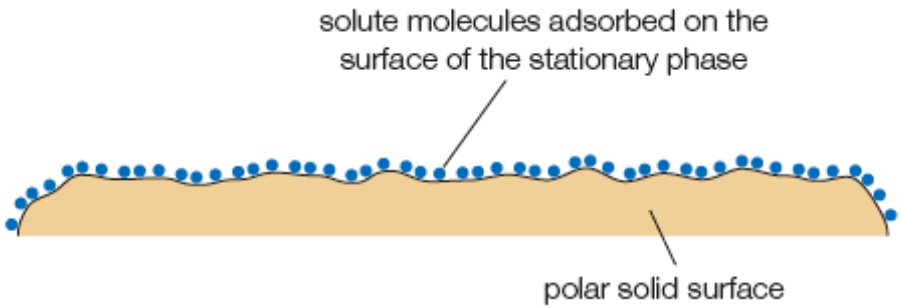
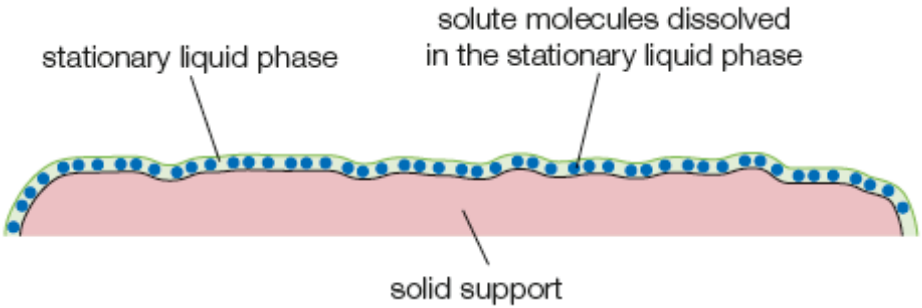
Solute Interactions





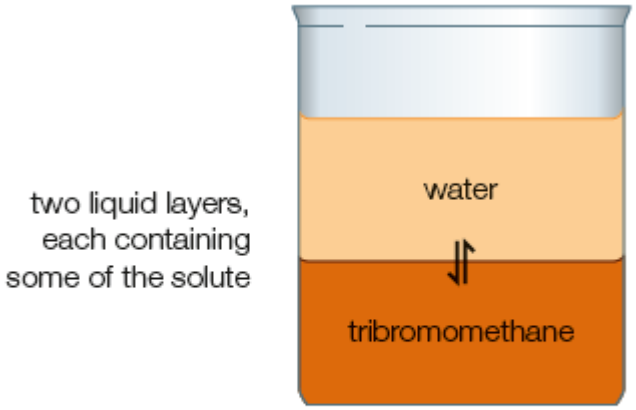


Figure 21.79 Bromine partitioned between water and tribromomethane

Adsorption

- Electrostatic interactions between sample and surface
- Solute molecules (polar molecules) are held on the surface of the stationary phase (polar solid)
- Separation of the solute depends on their polarity

Partition

- Solutes move between the stationary and mobile phase and are partitioned between them

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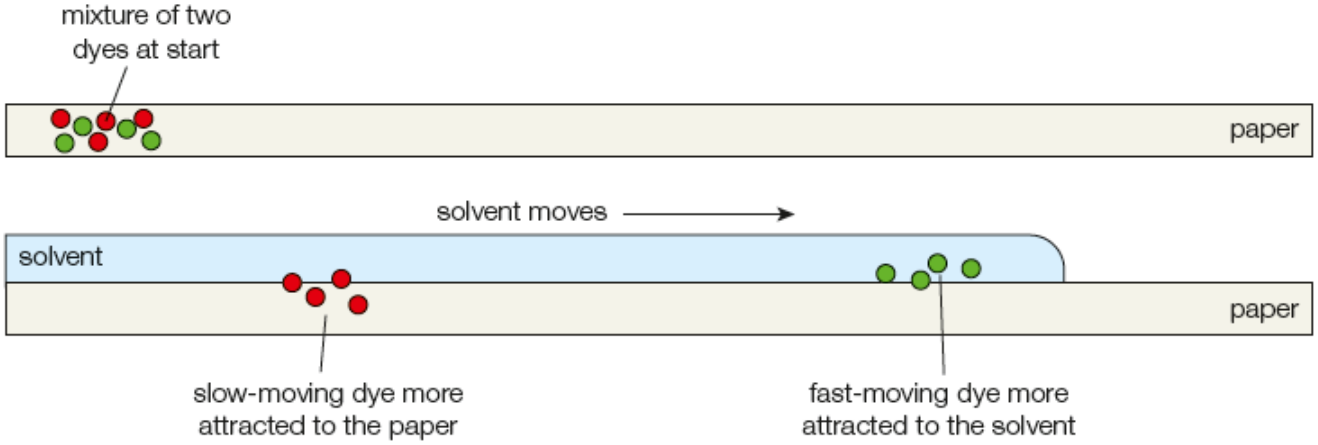
Paper, TLC & Column

A.7.3 Outline the use of paper chromatography, thin-layer chromatography (TLC) and column chromatography. (2)

- Paper Chromatography:
  - Uses: Smallest of the techniques, to separate dyes and identify biological molecules
  - Stationary Phase = H_2O molecules trapped in the water
  - Mobile Phase = aqueous or organic solvent that moves up the paper via capillary action
    - Dyes that are more soluble in the solvent than in the water of the stationary phase move rapidly up the paper
    - Dyes less soluble in the solvent remain in the stationary phase held to the water in the paper



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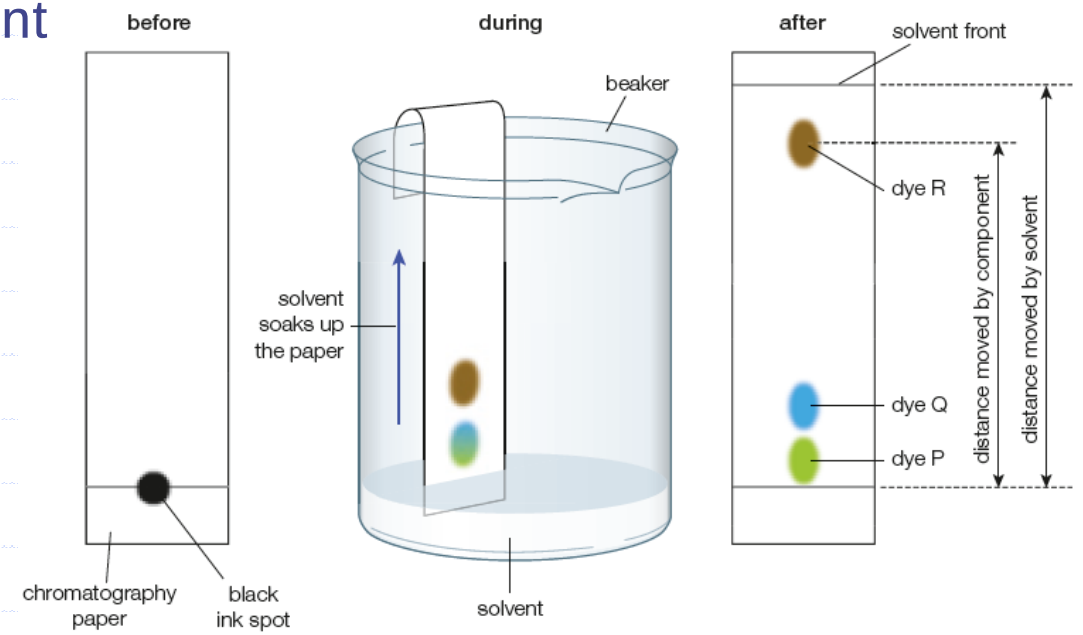
Paper Chromatography

- Paper is removed and left to dry and compared to the R_f value or **retention factor**
  - **Not** dependent on the distance travelled by the solvent but instead on the relation of the mobile to stationary phase
  - $R_f = \frac{distance\ moved\ by\ component}{distance\ moved\ by\ solvent}$
  - Compared to the R_f of known substances
  - Non-colored substances are treated with a dye (ex. Ninhydrin) as a locating agent



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Sugar Identification

- Allow the paper chromatogram to dry
- Pull through a solution of silver nitrate ($AgNO_3$) in aqueous Propanone (CH_3COCH_3).
- Solvent allowed to evaporate
- Paper sprayed with sodium hydroxide (NaOH) in aqueous ethanol (C_2H_5OH)
  - Reducing sugars (maltose/glucose): Black spots of silver

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TLC – Thin-Layer Chromatography

- Similar to Paper Chromatography but faster and works with smaller samples
  - Stationary phase is replaced by TLC plate on a class, aluminum foil or plastic plate
    - Silica (SiO_2)
    - Alumina (Al_2O_3)
  - Sample 'spotted' on the bottom of the plate
  - Plate placed in solvent (mobile) in a closed vessel to maintain a consistent atmosphere and minimize evaporation
  - View movement in visible light
  - View movement in UV light
  - View movement after dye is added (such as $KMnO_4$)

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Column Chromatography

- Allows for the large-scale separation of mixtures
- Process:
  - Glass column is ‘packed’ with an inert substance (silica or alumina again) which acts as the mobile phase
  - Mixture is added to the top of the column and allowed to run via the force of gravity
  - Components separate and arrive at bottom at different rates

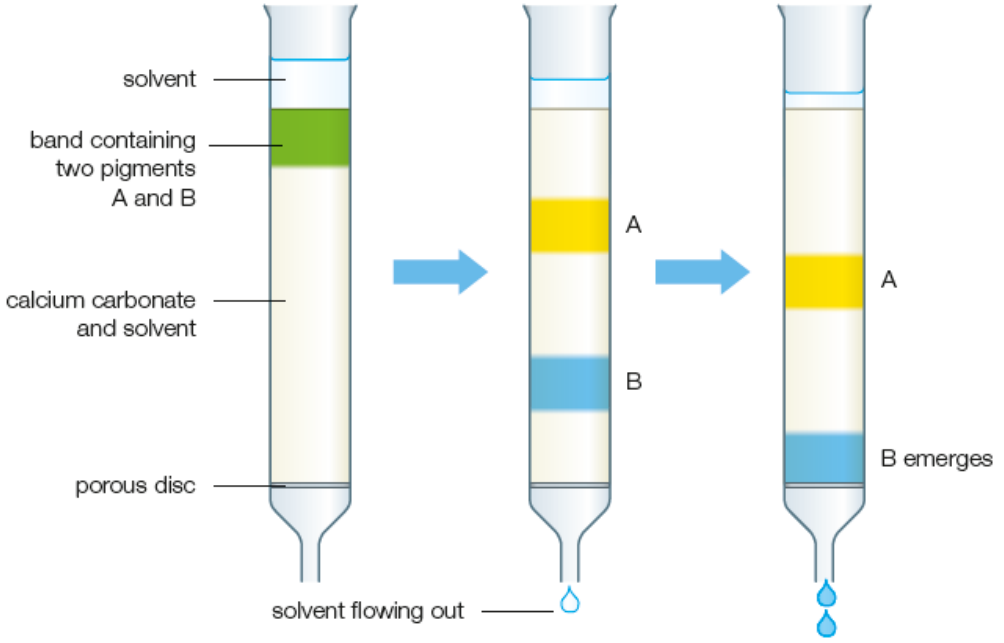

Figure 21.87 Separation of the components in plant pigments during column chromatography